Brief description of the drawings and tables

Table 1

Sequence of the primers used for genomic typing of HA-1 alleles by sequence-specific amplification.

Table 2

Sequence of the primers and probes used for genomic typing of HA-1 alleles by amplification and sequence-specific hybridization.

Table 3

Cellular and genomic typing for HA-1 in three HLA-A*0201 positive families

Table 4.

Comparison of cellular and genomic typing by PCR or LiPA of HA-1 in family 1.

Table 5. (Sequence ID Nos.: 25-28)

KIAA0223 sequence polymorphism in mH HA-1 positive and HA-1 negative individuals. Sequencing of HA-1 region in KIAA0223 gene in HA-1 +/+ and HA-1-/- homozygous individuals and KG-1 revealed two alleles differing in two nucleotides resulting in a one amino acid difference (H to R) and designated HA-1^{H} and HA-1^{R} . For DH and vR 6 independent PCR products were sequenced. For KG-1 8 PCR products were sequenced.

Figure 1. Reconstitution of HA-1 with HPLC fractionated peptides eluted from HLA-A2.1 molecules in a ^{51}Cr-release assay with mH HA-1 specific T cell clone 3HA15.

a. Peptides were eluted from 90.10^{9} HA-1 and HLA-A2.1 positive Rp cells and separated using reverse phase HPLC with HFBA as organic modifier.

b. Fraction 24 of the first HPLC dimension that contained HA-1 activity was further fractionated by reverse phase HPLC with TFA as organic modifier.

c. HA-1 containing fraction 27 of the second gradient was further chromatographed with a third shallower gradient consisting of 0.1 % acetonitrile/min. Background lysis of T2 by the

The clone Q66.9 is specific for the influenza matrix peptide 58-66. No TNFα production was observed after transfection of the pcDNA3.1(+) vector alone (results not shown).

Figure 4.

a. Binding of HA-1^H and HA-1^R peptides to HLA-A2.1. The binding of HA-1^H and HA-1^R peptides were assayed for their ability to inhibit the binding of fluorescent peptide FLPSDCFPSV to recombinant HLA-A2.1 and β2-microglobulin in a cell free peptide binding assay. One representative experiment is shown. The IC50 is determined on the results of 4 experiments and was 30 nM for VLHDDLLEA and 365 nM for VLRDDLLEA.

b. Reconstitution assay with different concentrations of synthetic HA-1^R peptide with HA-1 specific T cells. The HA-1^R peptide was titrated and preincubated with T2 cells. Three HA-1 specific T cell clones, 5W38, 3HA15 and clone 15 were added and a 4 hr ^{51}Cr-release assay was performed. Background lysis of T2 by the CTL in the absence of any peptide was for 3HA15 4%, for clone 15 10% and for 5W38 2%. Positive control lysis was for 3HA15 46%, for clone 15 47% and 5W38 48%.

Figure 5 (Sequence ID Nos.: 17-22)

Sequences and genomic structure of the HA-1 locus. Figure 1a, coding sequences of the H and R alleles of HA-1. Bold characters indicate the polymorphic nucleotides. Figure 1b, exon-intron boundaries of the HA-1 locus. Exon sequences are shown in uppercase, intron sequences in lowercase.

Figure 6

Genomic typing of HA-1 alleles in clinical samples. Genomic typing was performed by sequence-specific amplification, by use of the two primer sets of Table 1. The two upper fragments in the gel originate from the H-allele, the two lower fragments from the R-allele.

Figure 7

HA-1 typing by LiPA of family 1

regression analysis with the prismgraph software. Synthetic peptides were manufactured on a Abimed 422 multiple peptide synthesizer (Abimed, Langenfeld, Germany) and were more than 90% pure as checked by reverse phase HPLC.

1.2.6. RT-PCR amplification and sequencing of KIAA0223 region coding for HA-1

Total or mRNA was prepared from BLCL using the RNAzol methode (Cinas/Biotecx Laboratories, Houston, TX) or according to manufacturer's instructions (QuickPrep mRNA purification Kit, Pharmacia Biotech). CDNA was synthesized with 1 μg RNA as template and with KIAA0223 based reverse primer 5'-GCTCCTGCATGACGCTCTGTCTGCA-3'[1]. To amplify the HA-1 region of KIAA0223 the following primers were used: Forward primer 5'-GACGTCGTCGAGGACATCTCCCAT-3'[2] and reverse primer 5'-GAAGGCCACAGCAATCGTCTCCAGG-3'[3]. Cycle parameters used were denaturation 95 °C, 1 min, annealing 58 °C, 1 min and extension 72 °C, 1 min (25 cycles). The PCR-products were purified using the Magic PCR-Preps DNA purification System (Promega) and direct cloned using the pMosBlue T-vector kit (Amersham LIFE SCIENCE). Six independent colonies from each individual were sequenced using the T7-sequencing kit (Pharmacia Biotech).

1.2.7. HA-1 allele specific PCR amplification

In the case of HA-1 allele specific PCR amplification, cDNA was synthesized as described above. A PCR amplification was performed with allele specific forward primers: for the HA-1^H allele primer H1: 5'-CCT-TGA-GAA-ACT-TAA-GGA-GTG-TGT-GCT-GCA-3'[4]. for the HA-1^R allele primer R1: 5'-CCT-TGA-GAA-ACT-TAA-GGA-GTG-TGT-GTT-GCG-3'[5] and for both reaction the reverse primer as described above was used. Cycle parameters used were denaturation 95 °C, 1 min, annealing 67 °C, 1 min and extension 72 °C, 1 min (25 cycles).

1.2.8. Cloning and expression of HA-1^H and HA-1^R allelic region of KIAA0223.

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1 (Sequence ID No.: 30)

2 (Sequence ID No.: 31)

3 (Sequence ID No.: 32)

4 (Sequence ID No.: 33)

5 (Sequence ID No.: 34)